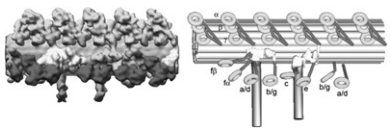
dyneins from *Chlamydomonas* stack vertically, while eight inner arm dyneins make a horizontal array (Ishikawa et al. (2007) JMB; Bui et al. (2008) JCB) (figure). We also found that the arrangement of inner dyneins and other linkers is not symmetrical among nine microtubule doublets (Bui et al. (2009) JCB). By further image analysis we revealed the shift of the ATPase head of dynein toward the tip of flagella during Pi release. The orientation of the coild-coil stalk is constant. This shift can winch adjacent microtubule. Interestingly apo and nucleotide-bound forms of dynein coexist and they make clusters in flagella, which could explain torsion for bending.



Platform AW: Protein Folding & Stability

3187-Plat
Computer Simulation Models of Protein Stabilization by Osmolytes
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Osmolytes are intracelular organic compounds that protect protein against unfolding in the presence of environmental stresses such as high temperatures, desiccations, or the presence of denaturants. In this work we examine the physics of protein stabilization by osmolytes with modified Go models. The reference Go model is a high-resolution Go model of the 20-residue Trp-cage protein (Linhananta et al., J. Chem. Phys. 122: 114901, 2005). Previously we showed that the Go model Trp-cage in vacuum exhibits cooperative behavior with a scaled folding temperature of $T^* = 4.0$. The model is generalized by immersing the protein in solutions of spherical solvent molecules whose interactions with the protein, controlled by the protein-solvent contact energy parameter, are adjusted to mimic the effects of osmolytes and urea solutions. For osmolyte solutions we setto mimic the repulsive interaction between osmolytes and proteins. Simulations of the models found the scaled folding temperature increases from the reference model value of $T^* = 4.0$ to $T^* > 5.5$. This demonstrates thermal stabilization by osmolytes, since the Trp-cage remains folded up to higher temperatures. We performed analysis to show that this stabilization arises from the osmolyte ability to reduce the entropy and free energy of unfolded states of proteins. Finally we calculate the cooperativity measure of the models to show that this stabilization occurs without any loss in cooperativity of the Go model protein.

3188-Plat
Citrate Binding Stabilizes Human Gamma-Crystallin to Slow Unfolding and Inhibit Aggregation
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Recent studies have demonstrated that small molecules can bind destabilized or aggregation prone proteins to prevent unfolding and aggregation. Cataract, the leading cause of blindness worldwide, is caused by aggregation of proteins in the eye lens. The most abundant proteins in the eye lens belongs to the βγ-Crystallin superfamily, which accounts for 90% of lens protein composition. These proteins are synthesized in utero and must remain stable and soluble throughout life. Damage, or deleterious post-translational modification can destabilize these proteins and induce aggregation-prone conformations. Sodium citrate has been shown to prevent unfolding and aggregation of alpha-antitrypsin by stabilizing secondary structure. In this study, we demonstrate the effects of citrate binding on both wild-type and disease models of Human γD Crystallin. Equilibrium unfolding-refolding experiments show an increase in the ΔG of unfolding with increasing concentrations of sodium citrate, while kinetic experiments show that sodium citrate slows the rate of unfolding in denaturant. UV resonance Raman spectroscopy has been used to examine Trp residues in the protein and monitor vibrational modes as a function of temperature. Preliminary results indicate a resistance to unfolding in the presence of citrate. The effect does not appear to be due to metal ion chelation, and may reflect direct binding to the crystallins, as with anti-trypsin.

3189-Plat
Peptide Folding on Peptide Amphiphile Micelles Determines Micelle Structure and Assembly
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Covalent attachment of a hydrophobic tail to a peptide produces chimeric molecules termed peptide amphiphiles (PAs). Difference in aqueous solubilities between the alkyl tails and peptide headgroup drives PA self-assembly in aqueous environment. We here show that self-assembly further induces peptide folding into secondary structure motifs because of peptide crowding in the micelle corona. Furthermore we present results to the effect that the type (alpha helix Vs beta sheets) and extent of folding is controlled by the chemistry of the linker between tail and the peptide headgroup.
We have prepared a series of peptide amphiphiles consisting of 1) a palmitic tail, 2) a bioactive, 16-amino acid peptide and 3) linkers differing in H-bonding potential, length and hydrophilicity. Circular dichroism and fluorescence spectroscopy were used to monitor shifts in secondary structure while dynamic light scattering, AFM and cryo-TEM provided information on supramolecular structure.
Our results demonstrate that H-bonding availability and linker length are determining factors for peptide folding upon PA self-assembly into worm-like micelles. Alpha helical content present in the control PA (direct amide bond linkage between peptide and tail) decreased with increasing ethylene oxide linker length. Instead, inclusion of 4 alanines as a linker promoted beta-sheet formation.
These changes in PA structure had an effect on micelle length and flexibility. Additionally, in presence of divalent Mg2+ ions elongation or stacking of worm-like structures was observed, when alpha helices and beta sheets were formed respectively.
Our results provide insight on the mechanisms through which headgroup structuring occurs on peptide-based micelles, with implications on the bioactivity, stability and morphology of the self-assembled entities.

3190-Plat
Millisecond Timescale, Atomistic Protein Folding Simulations Yield a Network Theory for Protein Folding
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Understanding protein folding is a classic grand challenge in molecular biophysics; a solution for which could have immediate medical benefits, particularly for protein misfolding diseases like Alzheimer's. Molecular Dynamics (MD) simulations have the potential to provide quantitative models of protein folding but, unfortunately, this potential has yet to be fully realized due to the need to capture long-timescale transitions at atomic resolution. Taking advantage of a new theory for molecular kinetics and the computational power of Graphics Processing Units (GPUs), however, we are now able to reach millisecond timescales at atomic resolution (one million times longer than conventional simulations). But, how can one use these simulations to gain insight? We present a novel network theory which is capable of quantitative prediction of the native states and folding timescales for the villin headpiece and NTL9, which fold on microsecond and millisecond timescales respectively. Furthermore, it leads to experimentally testable hypotheses about the nature of protein free energy landscapes and how proteins fold so quickly. We also reduce these concepts to simpler and more fundamental, humanly comprehensible networks that capture the essence of molecular kinetics and reproduce qualitative phenomena like apparent two-state folding. Models at both the quantitative and qualitative levels are crucial for gaining an intuition for molecular kinetics and for ultimately answering the general question of ''how do proteins fold?''

3191-Plat
Universal Convergence of the Specific Volume Changes of Globular Proteins Upon Unfolding
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Both pressure and temperature are important environmental variables, and in order to obtain a complete understanding of the mechanisms of protein folding, it is necessary to determine how protein stability is dependent on these fundamental thermodynamic parameters. Although the temperature dependence of protein stability has been widely explored, the dependence of protein stability on pressure is not as well studied. In this paper, we report the results of the direct thermodynamic determination of the change in specific volume ($\Delta V/V$) upon protein unfolding, which defines the pressure dependence of protein stability, for five model proteins (ubiquitin, eglin c, ribonuclease A, lysozyme, and cytochrome C). We have shown that the specific volumetric changes upon unfolding for four of the proteins (ubiquitin, eglin c, ribonuclease A, lysozyme) appear to converge to a common value at high temperature. Analysis of various contributions to the change in volume upon protein unfolding allowed us to put forth the hypothesis that the change in volume due to hydration is very close to zero at this temperature, such that $\Delta V/V$ is defined largely by the total volume of cavities and voids within a protein, and that this is a universal property of all small globular proteins without prosthetic groups. To test this hypothesis, additional experiments were performed with variants of eglin c that had site-directed substitutions at two buried positions, in order to create an additional cavity in the protein core. The results of these experiments, coupled with the structural analysis of cytochrome c showing a lower packing density compared to the other four proteins, provided further support for the hypothesis. The deviation of the high-temperature ΔV value from the convergence value can be used to experimentally determine the size of the excess cavities in proteins.